Inheritance of Seed Dormancy in Weedy Rice

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ABSTRACT

Seed dormancy contributes to the adaptability of plants in nature and is of considerable importance in agriculture. The weedy rice (*Oryza sativa* L.) strains LD, SS18-2, and TKN12-2 and cultivar 'N22' were selected to investigate the inheritance of dormancy in controlled conditions. Initial investigations using intact seeds, caryopses, caryopses with pericarp/testa removed, and excised embryos demonstrated that seed dormancy was imposed by the hull in SS18-2 and TKN12-2, and by the hull and pericarp/testa in LD and N22. Seed dormancy at 0 d after harvest (DAH) was dominant with average degree of dominance (ADD) > 0.8 in the crosses with weedy strains. Dominance for duration of seed dormancy was incomplete when judged by days to 50% germination. Broad-sense heritability (h_b^2) for seed germination was lower at 0 DAH and highest at 20 DAH in all the crosses. The weedy strain-derived F_2 populations maintained a higher h_b^2 during afterripening. The effects of three and two major genes on seed germination at 20 DAH were detected in the SS18-2- and N22-derived F_2 populations, respectively. A positive ADD, a high h_b^2, and major gene effect for caryopsis germination at 0 DAH were detected only in the cross with LD. Seed or caryopsis dormancy was correlated with the characteristics awn and black hull or red pericarp colors in the SS18-2- or LD-derived F_2 populations. This research demonstrates that weedy rice provides ideal gene resources to elucidate mechanisms of dormancy and to improve resistance to preharvest sprouting.

Dormancy distributes germination with time and is critical for the survival of seed-bearing plants. In agriculture, dormancy has considerable importance as it relates to preharvest sprouting and the persistence of weed seeds in the soil. Preharvest sprouting is germination in the inflorescence after maturation of the crop, when moist conditions prevail or untimely rains occur. Resistance to preharvest sprouting is correlated with the level of dormancy in dry, mature seeds (Seshu and Sorrells, 1986). Dormancy is the temporary failure of a viable seed to germinate, after a specific length of time, in a particular set of environmental conditions that allow germination after the restrictive state has been terminated by either natural or artificial conditions (Simpson, 1990, p. 3–113). Germinability is used to describe the capacity of seeds in a population with dormancy for immediate, intermediate, or much delayed germination due to the internal conditions (Foley, 2001). Despite years of research on seed dormancy, mechanisms for the regulation of germinability are basically unknown (Foley, 2001; Koornneef et al., 2002). Cloning and characterizing genes that directly regulate germinability should provide new insight into seed dormancy and resistance to preharvest sprouting.

Rice germplasm (*Oryza* spp.) varies in degree of seed dormancy (Roberts, 1961a; Wu, 1978; Oka, 1988, p. 87–123; Siddique et al., 1988; Kalita et al., 1994; Rao, 1994), and cultivated rice (*O. sativa* L.) is well suited for cloning dormancy genes. Rice is a diploid, has a relatively small genome, is the base genome for comparative genetic investigations in grasses, and many genetic and molecular resources are publicly available (Gale et al., 1996). Also, rice is the only species in the family Poaceae for which the draft genome sequences have been published, and a more detailed genome sequence will be available soon (Goff et al., 2002; Yu et al., 2002). However, before pursuing a cloning strategy for dormancy genes, it is critical to select appropriate gene donors in the species.

There are two common ways by which seed dormancy is imposed: seed coverings (e.g., pericarp, testa, and in some cases the endosperm) and the embryo itself (Bewley and Black, 1994, p. 199–230). In grasses, the term *seed* is commonly used to describe the dispersal unit (Simpson, 1990, p. 3–113). An intact rice seed has a hard enclosure–hull outside the caryopsis. There is abundant evidence that seed dormancy in rice is imposed through the hull, pericarp/testa, or both (Roberts, 1961b; Seshu and Dadlani, 1991). The occurrence of embryo dormancy in rice has been suggested (Takahashi, 1962; Nair et al., 1965), but remains uncertain because some reports indicate that rice lacks embryo dormancy (Roberts, 1961b; Seshu and Sorrells, 1986; Seshu and Dadlani, 1991).

Rice geneticists have studied the inheritance of dormancy to introduce the trait in elite cultivars to impart resistance to preharvest sprouting. Most genetic research focused on hull-imposed dormancy in cultivated rice. Chang and Yen (1969) and Chang and Tagumpay (1973) concluded that the seed dormancy is a quantitative trait governed by polygenes with cumulative but unequal effects and is strongly affected by environmental conditions during seed development. Heritability for the trait was low (0.12 to 0.42) in their field experiments. Other researchers used a Mendelian method to identify dormancy genes from rice cultivars (Takahashi, 1962; Tripathi and Rao, 1982; Tomar, 1984; Das and Bhaduri, 1985; Seshu and Sorrells, 1986; Shenoy, 1993; Das, 1995). These investigators developed a few putative models consisting of one or two major genes plus minor genes to explain the inheritance of dormancy in cultivars. More recently, researchers have identified ≈20 putative quantitative trait loci for seed dormancy in rice using molecular markers (Wan et al., 1997; Lin et al., 1998; Cai and Morishima, 2000).

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Abbreviations: ADD, average degree of dominance; DAH, days after harvest; DAI, days after imbibition; h_b^2, broad-sense heritability; RH, relative humidity.

Weedy rice accompanies the cultivation of rice worldwide. Generally, seed dormancy is stronger in weedy rice than in cultivated rice (Oka, 1988, p. 87–123; Cho et al., 1995; Suh et al., 1997; Tang and Morishima, 1997). It is likely that weedy rice has novel alleles that influence germinability and are not present in domesticated rice. No research has been done on the genetic basis for seed dormancy in weedy rice. Therefore, before we pursue a map-based cloning strategy, we characterized the inheritance and variation of seed dormancy in selected weedy rice strains in controlled conditions.

MATERIALS AND METHODS

Plant Materials

Four pairs of dormant and nondormant lines chosen as parents for this research were selected from 40 weedy and cultivated rice accessions based on their extreme phenotypes for germinability of intact seeds (data not shown). The dormant lines included two indica-type weedy strains SS18-2 and TKN12-2, a japonica-type weedy strain LD, and an indica-type traditional cultivar N22. The weedy strains were purified by self-pollination and single-plant selection for two or more generations before hybridization. The cultivar N22 was selected because, of the cultivars, its seeds and caryopses (hull removed) displayed the strongest dormancy. The nondormant lines included indica cultivars 'CO39' and 'Dular', an indica breeding line EM93-1, and a japonica cultivar 'WYJ'. Five crosses, including a set of reciprocal crosses between CO39 and SS18-2, were made to develop F_1 and F_2 generations.

Plant Cultivation and Seed Harvest

Afterripened seeds or caryopses from parental and F_1 plants were incubated at 35°C for 5 d to induce germination. Young seedlings were cultured in nutrient solution (Yoshida et al., 1976) for 25 d and then transplanted into 22-cm pots containing a greenhouse soil mixture. Plants were maintained in a large greenhouse with a day/night temperature set at 29/21°C. Supplementary light was used as needed to maintain a 14-h photoperiod, except at the late tillering stage when a 10-h photoperiod was used to induce and synchronize flowering for the weedy strain-derived populations. Flowering date for a plant was marked by emergence of the first panicle from the leaf sheath. Panicles in weedy strain-derived populations were covered with paper bags at ≈10 d after flowering. Bagged panicles were fixed to bamboo poles to prevent shattering during seed development due to brushing or shaking the plant. Seeds were harvested at 40 d after flowering. Freshly harvested seeds were cleaned by removal of green or not fully matured seeds. Cleaned seeds were put in paper bags and left at room temperature (≈25°C) for 3 d to dry. Dried seeds were stored at −20°C to maintain dormancy or afterripened at room temperature for various DAH.

Evaluation of Germination

To evaluate the influence of tissue components on seed dormancy in the parental lines, the germination of intact seeds, caryopses, caryopses with the pericarp/testa removed, and excised embryos was evaluated. Caryopses and pericarp/testa-removed caryopses were obtained by removing the hull by hand and by scraping the pericarp/testa with a razor blade after hull removal, respectively (Seshu and Sorrells, 1986). Embryos were excised with the aid of a surgical knife under a stereomicroscope (Foley, 1992). Twenty-four seeds or caryopses were placed in a 24-well tissue culture plate lined with a Whatman No. 1 filter paper and wetted with 0.25 mL of sterile water. There were three replications per treatment, and the experiment was repeated at least twice. Twenty excised embryos with three replications per treatment were cultured on N6 medium (Foley, 1992). The seeds, caryopses, and embryos were incubated in the dark at 30°C and 100% relative humidity (RH). Germination was rated every day for the first 7 d after imbibition (DAI) and then every other day for up to 28 d. Germination was judged when the radicle or coleoptile expanded by >3 mm. Percentage germination was used to evaluate the degree of dormancy.

Germination data from individual plants were used for genetic analyses. Seed germination was done at different DAH. The DAH or afterripening intervals were selected based on reports in the literature (Seshu and Sorrells, 1986) and our preliminary experiment using the seeds from parental and F_1 plants of each cross. Forty to 50 seeds of two replications each were placed in 9-cm Petri dishes lined with a Whatman No. 1 filter paper and wetted with 10 mL of deionized water, and incubated in the dark at 30°C and 100% RH. Caryopsis germination was evaluated only at 0 DAH. Thirty caryopses with three replications each were germinated in the same conditions as above. The sample size of individual parental and F_1 plants was 25 to 30. The sample size for SS18-2-, N22-, and LD-derived F_2 populations was about 200, 200, and 140 plants, respectively. Because of partial sterility in the LD/WYJ F_2 population, only half of the plants yielded sufficient seed for the seed germination assays at 20, 40, and 60 DAH.

Estimation of Genetic Parameters, Genetic Models, and Linkage

Seed germination at different DAH showed various patterns of continuous distribution. Therefore, two parameters, ADD and h_b^2, were used to compare the genetic difference in degree of dormancy between crosses and between germination assays at different DAH. The parameters were calculated based on principles described by Mather and Jinks (1971, p. 65–171):

$$\text{ADD} = [M_{F1} - (M_{PD} + M_{PND})/2]/(M_{PD} - M_{PND})/2,$$

and

$$h_b^2 = [V_{F2} - (V_{PD} + V_{PND} + V_{F1})/3]/V_{F2},$$

where M_{PD} and V_{PD}, M_{PND} and V_{PND}, M_{F1} and V_{F1}, and V_{F2} stand for means and variances of germination percentages in parental (dormant and nondormant), F_1, and F_2 generations, respectively. The M_{F1} and V_{F1} for the SS18-2/CO39 cross were replaced with those from its reciprocal cross because there was an insufficient amount of hybrid F_1 seeds. Germination data were transformed by $\sin^{-1}(\chi)^{-0.5}$ when the F_2 distribution skewed to the low or high ends of the percentage scale.

A genetic model for major dormancy genes was determined when an F_2 population clearly showed a bimodal distribution in a set of germination tests and a high h_b^2. The fitness of the models was tested by Chi-square.

Weedy rice is characterized by black hull and red pericarp colors, or long awns. Some morphological characters of the grain, such as the grain type in wild oat and the red seed coat color in wheat, influence seed dormancy (Johnson, 1935; Gfeller and Svejda, 1960; Flintham, 1992). Thus, the association between germinability and morphological characters in weedy strain-derived populations was evaluated using linear correlation analysis. These characters displayed the dominant phenotypes in F_1 generation. Therefore, for each characteristic, F_2 plants were simply classified into dominant and recessive groups. The presence of an awn, black hull color, and red pericarp coloration was designated as 1 and the absence as 0.

RESULTS

Types of Seed Dormancy

Dormant and nondormant lines differed in seed or caryopsis germination (Table 1). Nonafterripened (0 DAH) seeds and caryopses from the four nondormant lines displayed >81% and 94% germination, respectively, at 7 DAI. Germination of intact seeds increased to >90% by afterripening for an additional 3 d past their normal drying period (data not shown). This suggests that there is either a very low level of hull-imposed dormancy in these lines or >3 d are required to bring the water content of intact seeds to a level that will facilitate rapid and uniform germination on initial imbibition (Kermode, 1995).

Nonafterripened seeds of the four relatively dormant lines displayed <4% germination at 7 DAI (Table 1). Seed germination for the dormant lines remained unchanged following an additional 3-wk period of incubation. Nonafterripened caryopses of the four dormant lines displayed three levels of germinability. Caryopses of lines SS18-2 and TKN12-2 had weak, N22 had moderate, and LD had strong dormancy based on germination percentages at 7 DAI. The three levels of caryopsis dormancy were distinguishable even after an additional 3 wk of incubation (Table 1). Caryopses with the pericarp/testa removed and excised embryos displayed 95 to 100% germination at 7 and 4 DAI, respectively (Table 1). This rapid germination implies that neither endosperm- nor embryo-imposed dormancy occurred in these lines. Thus, caryopsis dormancy is imposed by the pericarp/testa, and dormancy with the intact seeds is imposed by both the hull and pericarp/testa. The pericarp and testa are grouped because they cannot be readily separated to examine their individual contribution to seed or caryopsis dormancy.

Table 1. Germination of parental line seeds, caryopses (I), and pericarp/testa-removed caryopses (II) at 7 and/or 28 d, and excised embryos at 4 d after imbibition (DAI) at 30°C.

Genotype	Seeds‡	Caryopses (I)‡	Caryopses (II)	Embryos
LD†	0 (0)	18 (27)	98	100
'N22'†	3 (3)	37 (43)	100	100
SS18-2†	1 (2)	83 (93)	100	100
TKN12-2†	0 (0)	77 (91)	95	100
'CO39'	81 (85)	95		
'Dular'	88 (92)	96		
EM93-1	83 (83)	96		
'WYJ'	85 (88)	94		

† Genotypes were used as dormant parents in this research.
‡ Numbers in parentheses are percentage germination at 28 DAI.

Since there was no detectable difference in the germination of intact nonafterripened seeds among the dormant lines, we determined their germination following various periods of afterripening. Using this technique, significant differences were detected in the number of days required to achieve an estimated level of 50% germination of intact seeds (Fig. 1). Forty, 55, 60, and

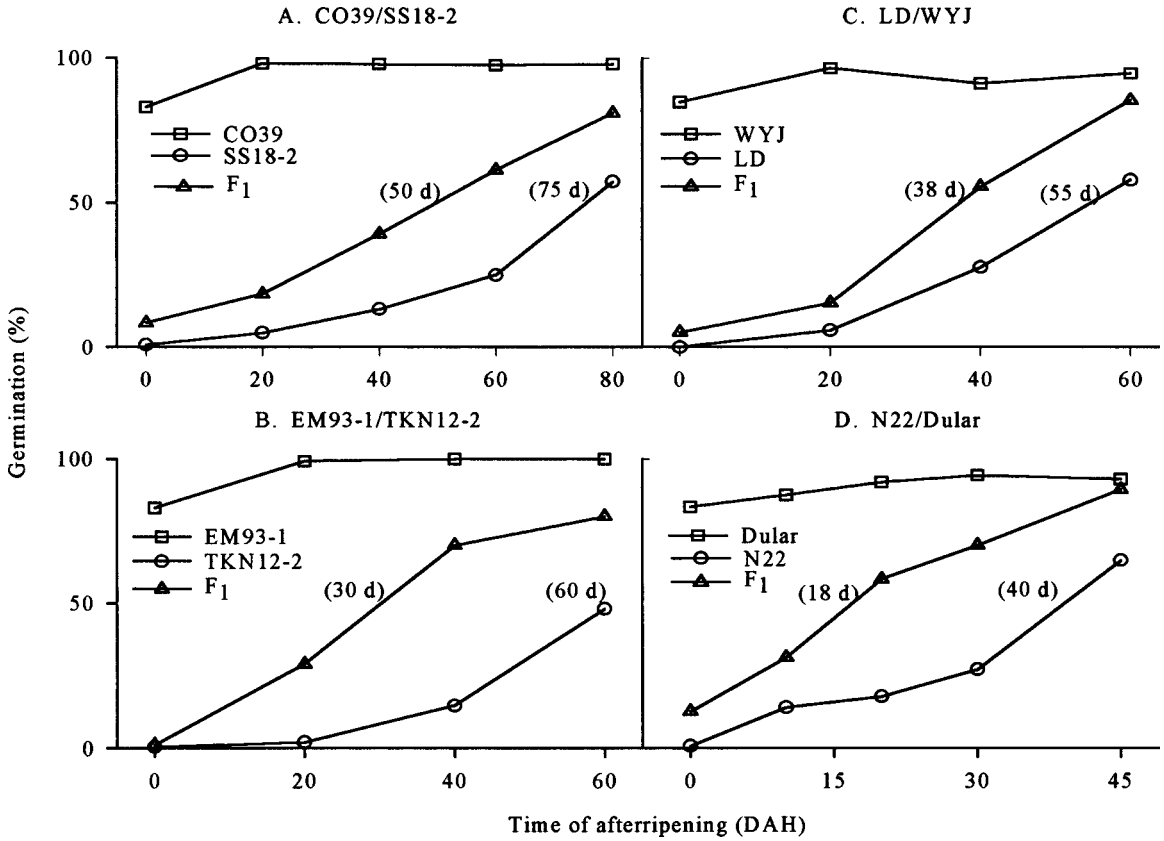

Fig. 1. Seed germination at 7 d after imbibition for dormant and nondormant parents and F_1 generation in relation to the period of afterripening or days after harvest (DAH) for four crosses. Numbers in parentheses denote the estimated days of afterripening required to achieve 50% germination for the dormant parent and F_1 generation.

Table 2. Average degree of dominance (ADD) for germination of seeds and caryopses based on the means of parental and F_1 generations.

Cross	Seeds at days after harvest								Nonafterripened caryopses
	0	10	20	30	40	45	60	80	
	ADD								
'CO39'/SS18-2	0.81		0.71		0.39		0.00	−0.16	−0.75
LD/'WYJ'	0.88		0.79		0.12		−0.50		0.90
EM93-1/TKN12-2	0.98		0.44		−0.29		−0.40		−0.12
'N22'/'Dular'	0.71	0.53	0.11	−0.28		−0.74			−0.27

75 d of afterripening were required to achieve 50% germination for N22, LD, TKN12-2, and SS18-2, respectively. There does not appear to be an association between the degree of caryopsis dormancy and the duration of dormancy with intact seeds among the dormant lines. For example, line SS18-2 had weak pericarp-imposed dormancy (Table 1) but had the longest duration of dormancy for intact seeds (Fig. 1). On the basis of the relative degree of pericarp-imposed dormancy and the duration of dormancy with intact seeds, three groups of dormancy genotypes were determined among the four dormant lines: (i) SS18-2 and TKN12-2 have weak caryopsis dormancy and a long duration of seed dormancy; (ii) N22 has a moderate level of caryopsis dormancy and a relatively short duration of seed dormancy, and (iii) LD has strong caryopsis dormancy and a moderate duration of seed dormancy.

Dominance

Dominance of seed dormancy varied with the period of afterripening and the measurement. The germination level of seeds at 0 DAH from F_1 plants was similar to that from their dormant parents (Fig. 1). The dormancy with the nonafterripened intact seeds appeared dominant over nondormancy, as observed in the crosses utilized by Tomar (1984), Seshu and Sorrells (1986), and Shenoy (1993). However, the germination for partially afterripened seeds showed large phenotypic difference between the homozygous and heterozygous genotypes. The ADD changed from >0.7 (highly positive) to 0 (no dominance), then to <0 (negative partial dominance) during afterripening (Table 2). The longer the duration of dormancy for a dormant parent, the longer the period of afterripening required by the seeds from the F_1 plants to change from positive to negative dominance. On the other hand, the period of afterripening required for seeds from F_1 plants to achieve 50% germination was intermediate between the dormant and nondormant parents (Fig. 1). Both the change of ADD with duration of afterripening or the intermediate level of germination in the F_1 generation are indicative that the dominance for duration of seed dormancy is incomplete.

Dominance of caryopsis dormancy varied with the degree of pericarp-imposed dormancy of the dormant parents (Table 2). For example, the ADD for LD/WYJ cross was highly positive, while the remaining three crosses exhibited different levels of negative dominance. In contrast with seed morphological or yield traits, research on seed dormancy is more difficult because the phenotype is strongly affected by the type of dormancy, the dormancy measure, and the duration of seed afterripening.

Heritability

Broad-sense heritability for intact seed germination varied with the cross and the duration of afterripening (Table 3). Heritability at 0 DAH ranged from 0.64 to 0.76 for four F_2 populations. At 20 DAH, h_b^2 increase to >0.84. For seed germination of the F_2 populations from the N22/Dular cross at 45 DAH and the LD/WYJ cross at 60 DAH, h_b^2 was 0, indicating that most individuals lost dormancy, or genetic variation in seed dormancy cannot be detected in the populations at these stages. The F_2 populations derived from SS18-2, the parent with the longest duration of seed dormancy, maintained a higher h_b^2 during the 60-d period of afterripening.

Heritability was much higher for caryopsis germination in the F_2 population from the LD/WYJ cross than in the remaining two populations (Table 3). These data from the F_1 and F_2 generations support a large genetic differentiation between the dormant lines for pericarp-imposed dormancy. Heritability was higher for caryopsis germination (0.82) than for seed germination (0.76) at 0 DAH in the LD-derived F_2 population (Table 3). The main reason is that most F_2 plants had very strong dormancy with nonafterripened seeds leading to a more skew and narrower distribution, and the removal of the hull released part of the seed dormancy, resulting in a relatively even distribution across the percentage germi-

Table 3. Broad-sense heritability (h_b^2) for seed and caryopsis germination at different days after harvest (DAH) based on the variances of parental, F_1, and F_2 generations.

Cross	Seeds at DAH							Caryopses at 0 DAH
	0	10	20	30	40	45	60	
	h_b^2							
'CO39'/SS18-2	0.64†		0.93		0.82		0.84	0.34†
SS18-2/'CO39'	0.67†		0.95		0.83		0.83	
LD/'WYJ'	0.76†		0.95		0.55		0.00	0.82†
'N22'/'Dular'	0.73†	0.80	0.85	0.74		0.00		0.43†

† Values are calculated with the germination data transformed by $\sin^{-1}(\chi)^{-0.5}$.

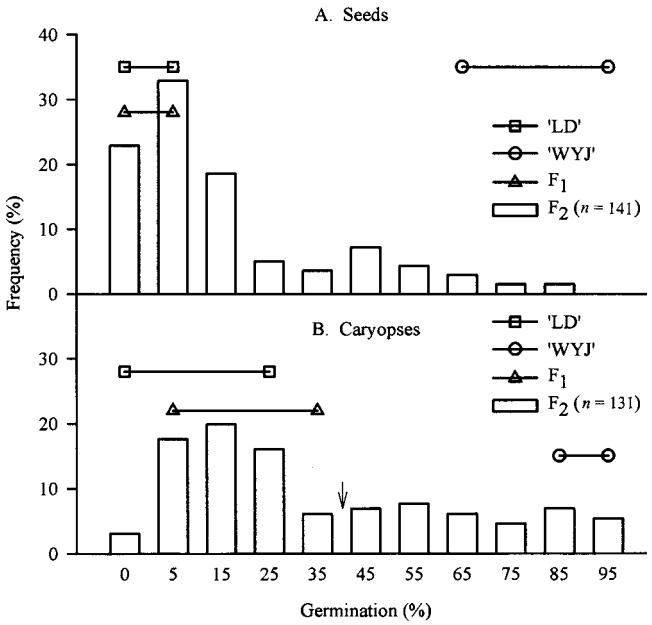

Fig. 2. Distribution of the F_2 population and range of parental and F_1 generations from the LD/'WYJ' cross for nonafterripened seed (A) and caryopsis (B) germination at 7 d after imbibition. The arrow is an assumed boundary of 40% segregating for the groups with lower and higher germination.

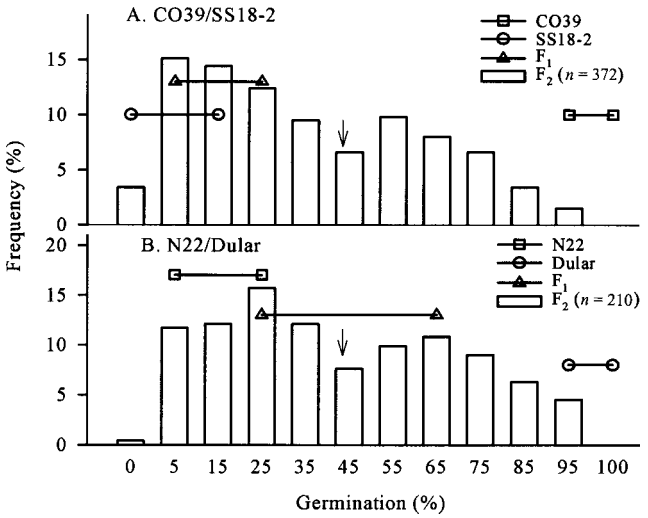

Fig. 3. Distribution of the F_2 population and range of parental and F_1 generations from the crosses of 'CO39'/SS18-2 (A) and 'N22'/'Dular' (B) for germination at 7 d after imbibition of seeds at 20 d after harvest. The arrow is an assumed boundary of 45% segregating for the groups with lower and higher germination.

nation scale (Fig. 2A and B). Basically, when dormancy is too strong, the percentage germination cannot be used to reflect the genotypic difference.

F_2 Segregation Patterns

A bimodal distribution for seed germination occurred in the N22- and SS18-2-derived F_2 populations at 20 DAH, with a natural boundary for low and high germination groups at ≈45% (Fig. 3A and B). The distribution in Fig. 3A is based on data from the SS18-2-derived reciprocal F_2s. The data were merged because the population means, variances, and distribution patterns in a set of germination assays are not significantly different (data not shown). The bimodal distribution indicates that there are major genes for seed dormancy in N22 and SS18-2. The ratio of the low- and high-germination plants in the N22/Dular cross was 125:98, fitting a digenic model of 9:7 ($P = 0.95$). According to the digenic model, the F_1 hybrid (*D1d1D2d2*) should distribute in the low germination group. In fact, the F_1 distribution for seed germination at 20 DAH ranged from 30 to 65%, crossing over the segregation boundary (Fig. 3B). A similar phenomenon occurred in three other F_2 populations based on which mono- and digenic models were proposed (Seshu and Sorrells, 1986). Therefore, there must be other modifiers that cause an increase in the length of dormancy or a decrease in the level of germination of the dormant genotypes in the low germination groups.

As compared with the N22-derived F_2 population at 20 DAH, the SS18-2-derived F_2 population had more plants in the low germination group and the modes for the low and high germination groups were lower (Fig. 3). The comparison strongly indicates the involvement of additional dormancy gene(s) controlling dormancy in SS18-2. Out of the 372 F_2 individuals, there were six plants that had a germination level of >90% at 20 DAH, which was the same as the nondormant parent CO39 (Fig. 3A). This proportion (6/372 = 1.61%) is very near to 1/64 (1.56%), the expected ratio for the homozygous nondormancy genotype at three loci in an F_2 population. Thus, we introduced another gene, *d3* for hull-imposed dormancy, to the digenic model for the N22-derived F_2 population to explain the major gene effect revealed by the distribution pattern for SS18-2-derived F_2 population. We assumed that *d3* is a recessive dormancy allele and alleles *D1* and/or *D2* are epistatic to *D3*. Furthermore, the gene interlocus interaction effect of seed dormancy alleles at three loci (e.g., *D1_D2_d3d3*) on dormancy is larger than that of dormancy alleles at any two loci (e.g., *D1_D2_D3_*, *D1_d2d2d3d3, d1d1D2_d3d3*), and the aforementioned interlocus interactions contribute to the higher level of dormancy or the lower level of seed germination at this stage. In fact, the dominant allele *D3* has a function similar to the afterripening promoting allele *E* in Jana's three-locus model for seed dormancy in wild oat (Jana et al., 1979, 1988). On the basis of the assumptions, the expected frequencies of the genotypes in the low germination group are 42/64, the remaining genotypes would belong to the higher germination groups that have moderate to low levels of dormancy or are nondormant. The observed ratios of the plants in the low and high germination groups in the F_2 population was 239:133, fitting the ratio of 42:22 ($P = 0.57$).

Seed germination was weakly correlated to the caryopsis germination in the N22- and SS18-2-derived F_2 populations (Table 4). The weak correlation ($r^2 < 4\%$) suggests that the aforementioned putative major genes are responsible for the hull-imposed dormancy.

A high correlation between caryopsis and seed germination was observed in the F_2 population derived from LD (Table 4), the weedy strain with strong dormancy

Table 4. Linear correlation coefficients between the germination of nonafterripened caryopses and the germination of seeds at different days after harvest (DAH) in F_2 populations.

F_2 populations	0 DAH	20 DAH	40 DAH	60 DAH
LD/'WYJ'	0.6513**	0.5215**	0.3455**	0.2262
SS18-2/'CO39'	0.1639*	0.2043**	0.1804*	0.1218
'N22'/'Dular'	0.1897**	0.1860**		

* Indicates significance at $P = 0.05$.
** Indicates significance at $P = 0.01$.

imposed by pericarp (Table 1). The high correlation (e.g., $r^2 = 42\%$ at 0 DAH) indicates that the genetic variation in seed germination arose from the confounding effect of the hull- and pericarp-imposed dormancy. However, the genetic variation in caryopsis germination can be merely explained by pericarp-imposed dormancy in the population given the absence of endosperm- and embryo-imposed dormancy (Table 1). F_2 plants, with respect to germination of nonafterripened caryopses at 7 DAI, can be categorized into two groups with a boundary at 40% (Fig. 2B). The group of plants with lower germination was similar to the dormant parent or F_1 plants in their level of caryopsis dormancy. The group with higher germination included the plants with moderate levels of caryopsis dormancy and the nondormant individuals (Fig. 2B). The ratio of the plants in the two groups was 82:49, which fits the trigenic ratio of 42:22 ($P = 0.46$). Incubation for an additional 2 wk increased the mean caryopsis germination of the population by 5%. This caused the boundary to move to 45%, but it did not change the segregation ratio (data not shown).

Association between Morphological Characteristics and Dormancy

Linear correlation analysis revealed an association between morphological characteristics and seed or caryopsis germination in the weedy strain-derived F_2 populations (Table 5). The presence of an awn was negatively correlated with seed germination in the LD- and SS18-2-derived F_2 populations. Black hull color was negatively correlated with seed germination in the SS18-2-derived F_2 populations and with caryopsis germination in the LD-derived F_2 population. Red pericarp color had a negative correlation with caryopsis germination in the LD-derived F_2 population (Table 5). Similar to the seed shattering in the collection of weedy rice (Oka, 1988, p. 113), the grain type in wild oat (Johnson, 1935), and the red pericarp color in wheat (Gfeller and Svejda, 1960; Flintham, 1992), the above character forms tend to correlate with the degree of seed or caryopsis dormancy. The presence of an awn, black hull color, and red pericarp color was dominant over the absence of these characteristics in the F_1 generation of the crosses. However, individuals with the dominant phenotype in the F_2 populations differed in the degree of trait expression. For example, the mean length of awns in a panicle varied from 1 to 8 cm, and the percentage of seeds with an awn in a panicle varied from a few to 100%. A range of variation suggests that more than one gene controls the morphological characteristics in weedy strains. Therefore, it is reasonable to attribute the association observed in the F_2 populations to the linkage of some of genes for dormancy with some genes for the morphological characteristics.

DISCUSSION

Investigating seed dormancy has been challenging because seed tissue components are genetically different in generation and ploidy (Johnson, 1935). The hull, pericarp, and testa are diploid maternal tissues while the embryo and endosperm are diploid and triploid offspring tissues. Embryo dormancy was reported in wild oat (*Avena fatua* L.) (Black, 1959; Simpson, 1965; Foley 1992). Therefore, the genetic research was based on the offspring generation (embryo genotype). These analyses resulted in the development of the three-locus model for dormancy in wild oat (Johnson, 1935; Jana et al., 1979; Jana et al., 1988; Fennimore et al., 1999). In rice, Takahashi (1962) observed differences in percentage germination of excised embryos between dormant and nondormant strains at 4 d after incubation. Research on dormancy in rice that used an offspring generation approach seemed to be unable to explain the experimental results based on the embryo genotype (Nair et al., 1965; Mitra et al., 1975). The putative *dominance* for dormancy differed between reciprocal hybrid F_1 seeds. This kind of result can be explained by an effect of maternal tissues (Seshu and Sorrells, 1986). We did not detect a significant effect of the embryo or endosperm

Table 5. Linear correlation coefficients between the morphological characters and the germination of intact seeds and caryopses in weedy strain-derived F_2 populations.

F_2 population	Characteristics	Seeds at DAH†				Caryopses at 0 DAH
		0	20	40	60	
'CO39'/SS18-2‡	Awn	−0.2120**	−0.2308**	−0.1788*	−0.2116**	−0.1176
	Black hull color	−0.2583**	−0.2988**	−0.3018**	−0.3076**	0.0018
	Red pericarp color	0.0028	0.0236	0.0905	0.0534	−0.091
SS18-2‡/'CO39'	Awn	−0.2284*	−0.2800**	−0.2278**	−0.1574*	−0.1089
	Black hull color	−0.2436**	−0.2013**	−0.2062**	−0.1623*	0.0638
	Red pericarp color	0.0179	0.0069	0.0798	0.0647	−0.083
LD/'WYJ'‡	Awn	−0.1190	−0.2190*	−0.1716*	−0.1632	−0.1497
	Black hull color	−0.0211	−0.0925	−0.0204	−0.0050	−0.1760*
	Red pericarp color	−0.1591	−0.1341	−0.1288	−0.1125	−0.2889**

* Indicates significance at $P = 0.05$.
** Indicates significance at $P = 0.01$.
† DAH = days after harvest.
‡ The parents have a long awn, black hull color, and red pericarp color; the absence and presence of the characteristics are designated as 0 and 1, respectively, in F_2 populations.

on germinability in the strongly dormant weedy rice strains (Table 1). Dormancy imposed by embryo and/or endosperm in rice either has not been detected or is not of sufficient strength in germplasm that has been used for genetic analyses to be distinguished from other genetic effects associated with the seed covering tissues. Hull- and pericarp-imposed dormancy has been well documented in cultivated (Roberts, 1961b, Nair et al., 1965; Chang and Yen, 1969; Chang and Tagumpay, 1973; Das, 1985; Seshu and Sorrells, 1986; Seshu and Dadlani, 1991) and wild rice (*Oryza* spp.) (Takahashi, 1962; Wu, 1978). We have detected hull- and pericarp-imposed dormancy in weedy strains of rice (Table 1), and therefore used a genetic approach based on the maternal generation rather than the offspring generation.

Hull-imposed dormancy in cultivated rice has been treated as a quantitative trait (Chang and Yen, 1969; Chang and Tagumpay, 1973). Many researchers emphasized the influence of environmental factors during seed development, storage, and germination on the trait (Ghosh, 1962; Roberts, 1962; Nair et al., 1965; Hayas and Hidaka, 1979; Rao 1994). We controlled environmental factors during plant development, afterripening, and germination. However, the metrical scale, that is, percentage germination, was an important factor affecting the phenotypic evaluation because many F_2 plants had a very high level of dormancy in the present experiment. We were not able to quantify differences between genotypes when the seeds were fully dormant. For example, ≈23% of the LD-derived F_2 plants displayed 0% germination at 0 DAH (Fig. 2A). Although these individuals were phenotypically identical at 0 DAH, they must contain different genotypes because their germination ranged from 0 to 40% when germinated at 20 DAH (data not shown). In this situation, genetic variance of the population would inevitably be underestimated because the individuals with 0% germination contributed relatively less to the genetic variance than if they displayed some level of germination. Since there is no reasonable scale transformation to correct this type of measurement error, we began to test germination using partially afterripened seeds (Tables 2 and 3).

Seed dormancy is a physiological trait. Its genetic behavior at the phenotypic level, such as dominance and heritability, varied not only by the parental genotypes and metrical scales but also depending on the period of afterripening (Tables 2 and 3). Rice breeders and geneticists have investigated the inheritance of seed dormancy for the purpose of breeding cultivars resistant to preharvest sprouting (Nair et al., 1965; Chang and Tagumpay, 1973; Seshu and Sorrells, 1986; Rao 1994). Knowledge about dominance and heritability is important to estimate selection efficiency in a breeding program. The heritability for dormancy in cultivated rice ranges from 0.12 to 0.42 (Chang and Yen, 1969). Weedy rice derived populations maintained a higher level of heritability for seed dormancy during a longer period after harvest as compared with the cultivar N22-derived population (Table 3). Thus, some strains of weedy rice should be good donors to impart resistance to preharvest sprouting in cultivated rice. Further study should be done to estimate the main genetic components contributing to the high genetic variation because the higher ADD at the early stages of afterripening (Table 2) implied the presence of gene nonadditive effects. Moreover, the basis for altered heritability in the same generation due to afterripening should be examined, as it is essentially different from the decrease in heritability as a generation is advanced.

Weedy rice harbors more major dormancy genes than cultivated rice. One or two genes for hull-imposed dormancy have been suggested in cultivars (Tripathi and Rao, 1982; Tomar 1984; Das and Bhaduri, 1985; Seshu and Sorrells, 1986; Shenoy, 1993; Das, 1995). On the basis of reciprocal F_2 germination data at 20 DAH, we proposed three major genes regulating hull-imposed dormancy in SS18-2. There must be other factors modifying the effect of the putative genes on germination of seeds before 20 DAH. Chang and Tagumpay (1973) postulated that more dominant alleles participate in the control of dormancy with nonafterripened seeds. Additional factors may relate to the physiological state of endosperm and embryo (Takahashi, 1962), or relate to the influence of the pericarp (Seshu and Sorrells, 1986). In the SS18-2-derived population, the weak correlation between seed and caryopsis germination (Table 4) suggests that the influence of the pericarp, endosperm, and embryo is not the main reason. It is possible that we have not yet detected all the genes that regulate hull-imposed dormancy.

There is no report in the literature concerning the genetic behavior for pericarp-imposed dormancy in domesticated and nondomesticated rice. The weedy strain LD displayed strong pericarp-imposed dormancy (Table 1). A major gene effect on the caryopsis dormancy was detected in LD (Fig. 2B), and based on the F_2 distribution at 0 DAH, we estimate the involvement of three major genes in the control of pericarp-imposed dormancy.

We observed more complex segregation patterns in F_2 populations because the parents have more dormant seeds or caryopses in our experiment as compared with previous genetic experiments. The three-gene model we postulated for pericarp- and hull-imposed dormancy is the simplest explanation for our data. The gene interlocus interaction revealed in our model is more important than the estimates of the number of major genes. We are developing backcross generations to expand on the putative models.

There is variation in seed dormancy in weedy rice (Cho et al., 1995; Suh et al., 1997; Tang and Morishima, 1997). The strains SS18-2 and LD are typical genotypes with hull- and pericarp-imposed dormancy, respectively. The genetic relationship between the two types of seed covering-imposed dormancy is unknown. Wu (1978) and Seshu and Sorrells (1986) postulated that dormancy due to the hull and pericarp is genetically independent. Our data indicated that germination of intact seeds was correlated with caryopsis germination, especially in the LD-derived F_2 population (Table 4). This correlation does not support the postulation that these two types of dormancy in rice are totally independent. It is likely that

some genes act on both types of dormancy while others act independently. The result from the correlation analysis is insufficient to determine the allelic relationship between genes that regulate seed and caryopsis dormancy in LD and SS18-2. Comparative genetic mapping using populations derived from genotypes with hull- and pericarp-imposed dormancy, respectively, will be necessary to further investigate the underlying bases for seed covering-imposed dormancy.

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